REMARKS/ARGUMENTS

I. Status of the Claims

Claims 58, 61-109 and 113-119 are pending in the application. Applicants seek to enter new claims 120 and 121 and cancel claims 62 and 63 without prejudice or disclaimer. Claim 58 has been amended to incorporate the limitations of dependent claims 62 and 63 with the proviso that when there is a translation start codon, there is no operably-linked stop codon in the exon. Support for this proviso can be found in Applicants' specification, *inter alia,* on page 38, construct (3). New claim 120 has been added which is directed to a host cell containing the originally-claimed vector of claim 58 with the proviso that endogenous protein expression is activated in the cell. Support for producing protein from a vector start codon and the endogenous gene can be found, *inter alia*, in Applicants' specification on page 38, construct (3). Such a construct inherently lacks an operably-linked stop codon. New claim 121 is also added. This claim corresponds to original claim 93. Accordingly, no new matter has been added with these amendments.

II. Reason for Amendment

In Applicants' Response dated July 23, 2003, Applicants amended claim 58 to distinguish the claim from Rust, *et al,*. Am. J. Respir. Cell Mol. Biol., 14:121-130 (1996). Rust disclosed a vector containing a selectable marker (ampicillin) expressed from its native promoter, the ampicillin promoter, which does not function in eukaryotic cells. Applicants seek to further amend claim 58 in view of reconsideration of a reference cited in Applicants' Information Disclosure Statement dated January 18, 2000. The reference, Burgess, *et al.*, is based on U.S.

Application No. 08/907,598. This corresponds to U.S. Patent No. 6,139,833. Burgess discloses a vector, VICTR 12 in Figure 2. This vector contains a PGK promoter operably linked to a puromycin gene (selectable marker) which is operably linked to a splice donor sequence. These components are integrated into both of the vector long terminal repeats. The puromycin gene, used as a selectable marker, contains both a translational start codon and an operably-linked translational stop codon. Therefore, Applicants have further amended claim 58 to recite that the exon either lacks a translational start codon or contains a translational start codon without an operably-linked translational stop codon.

Applicants also seek to add new claim 120, directed to a cell in which the integrated vector activates protein expression. Rust does not even teach a cell with an integrated vector. The Examiner also cited Hay, *et al.* (Proc. Natl. Acad. Sci. USA, 94:5195-5200 (1997), herein "Hay") against claim 58 in the Office Action dated April 23, 2003. The Hay vector also contains the ampicillin gene linked to its native promoter. Hay, likewise, does not even teach a cell in with an integrated vector.

Burgess does not teach the activation of protein expression following vector integration. Burgess does teach non-homologous vector integration and transcriptional activation, but teaches away from translation of the activated transcripts.

Burgess Does Not Disclose or Suggest
Protein Expression From An Endogenous Gene

Burgess produces a fusion *transcript* by splicing the vector splice donor onto the endogenous splice acceptor. Gene expression from the Burgess vectors was limited to the

nucleic acid level. Since Burgess was limited to expression of endogenous nucleic acid, there would have been no motivation to express a fusion protein from the selectable marker exon in the Burgess vector. Burgess actually teaches away from translation of the endogenous gene, as is discussed in detail immediately below.

Burgess Teaches Away From Protein Expression From An Endogenous Gene

The Burgess vectors at issue contain a selectable marker 3′ to a promoter and linked to a 3′ splice donor. The selectable marker lacks a poly A site. The poly A site is supplied by the trapped gene. When a gene is trapped, therefore, a fusion transcript is produced containing the selectable marker RNA and RNA from the trapped gene. The selectable marker RNA is translated, but the RNA from the endogenous gene is not translated. The purpose of producing RNA from the endogenous gene is to provide nucleic acid sequence information about the gene.

The goal of Burgess is to provide a "knock-out" mouse for genes in the genome that are not easily accessible and/or have DNA binding function (page 9, lines 1-14; page 13, lines 3-33). Towards this goal, the identity of the "knocked-out" gene is determined. Thus, the Burgess vectors provide two functions: "knock-out" and identification of a gene. Accordingly, the vectors contain sequences that disrupt expression of a gene and sequences that produce a fusion *transcript* between the marker and the endogenous gene. The disrupted gene is identified by endogenous gene sequences on the fusion transcript. Accordingly, identification is by nucleic acid sequencing only. For use of the fusion transcript, see Burgess, paragraph spanning pages 30-31; Figure 4 and figure legend; and Sections 5.5-5.7.

Thus, Burgess does not disclose or suggest the production of a protein from the endogenous gene.

On the contrary, Burgess specifically teaches away from producing protein from the endogenous gene.

> All of the gene trap vectors of the VICTR series, with the exception of VICTRs A and B, are designed to form a fusion transcript between vector encoded sequence and the trapped target gene. All of the flanking exons may be sequenced according to the methods described in the following section. To facilitate sequencing, specific sequences are engineered onto the ends of the selectable marker (e.g., puromycin coding region). Examples of such sequences include, but are not limited to unique sequences for priming PCR, and sequences complementary to standard M13 sequencing primers. **Additionally, stop codons are added in all three reading frames to ensure that no anomalous fusion proteins are produced.** All of the unique 3' primer sequences are immediately followed by a synthetic 9 base pair splice donor sequence. This keeps the size of the exon comprising the selectable marker at a minimum to ensure proper splicing, and positions the amplification and sequencing primers immediately adjacent to the flanking trapped exons to be sequenced as part of the generation of the collection of cells representing mutated transcription factor targets.

See Burgess, page 39, lines 16-35, emphasis added.

This shows that Burgess obtains expression of the endogenous gene only to obtain nucleic acid sequence information. Protein expression is neither disclosed nor suggested. In fact, Burgess teaches *preventing protein expression* from the endogenous gene to avoid problems with marker expression that could result from the production of anomalous fusion proteins produced by fusion of a marker protein with protein from the endogenous gene. Moreover, to express the endogenous protein would defeat the purpose, which was to knock-out endogenous

protein expression. Accordingly, Burgess teaches away from the production of endogenous protein sequences.

Applicants also seek to add new claim 121. This claim is also distinguished from Burgess, Hay and Rust for the reasons given above.

Accordingly, Applicants believe that this case is in condition for allowance. Early notification to that effect is requested. If the Examiner believes that a telephone conversation would expedite prosecution, he is invited to contact Applicants' attorney, Anne Brown, at 216-426-3586 or Cynthia L. Kanik, at 617-227-7400.

The Commissioner is hereby authorized to charge any fee deficiency to Deposit Account No. **50-2546**, referencing Attorney Docket No. **ATX-007CP4DV7**.

Respectfully submitted,

Anne Brown
Registration No. 36,463

Lahive & Cockfield
28 State Street, 24th Floor
Boston, MA 02109
Phone (617) 227-7400
Fax (617) 742-4214

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